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Mitochondrial Disease: mtDNA and Protein Segregation Mysteries in iPSCs

Mitochondrial diseases cause a range of clinical manifestations even in patients carrying the same mtDNA mutations. New work reveals that a common disease-associated mtDNA mutation is selectively segregated from wild-type mtDNA during the reprogramming of induced pluripotent stem cells and that high levels of this mutation in differentiated neurons upregulate Parkin-mediated mitophagy.

Alicia M. Pickrell and Richard J. Youle*

Multiple copies of mitochondrial DNA (mtDNA) reside in mitochondria and this DNA encodes the tRNA and rRNA machinery required to translate 13 components of all of the complexes of the respiratory chain, except complex II. Mutations in the mtDNA that affect oxidative phosphorylation function are common; it is estimated that 1 in 5,000 children and adults have mitochondrial diseases caused by mtDNA mutations [1]. MtDNA mutations reach varying levels of heteroplasmy — the ratio of wild-type to mutated mtDNA molecules — in different tissues and present a wide range of clinical symptoms, even between patients harboring the same type of mutation. The most common mtDNA mutation, m.3243A>G, disrupts the gene encoding tRNA Leucine$^{(UUR)}$ and causes two distinct mitochondrial diseases: maternally inherited diabetes and deafness (MIDD) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) [1]. To date, the most puzzling question is how mtDNA mutations affect different cell types to cause different phenotypes and how mutational load is determined in different tissues. Recent work by Hämäläinen *et al.* [2], published in *Proceedings of the National Academy of Sciences,* used an induced pluripotent stem cell (iPSC) model to provide mechanistic insight into how mtDNA mutations affect neurons differently from other cell types and how mtDNA mutations segregate in iPSCs to affect their differentiated progeny.

This study from Suomalainen's group shows that the m.3243A>G mutation causes a defect in respiratory chain complex I in differentiated neurons, but has no detrimental effect on oxidative phosphorylation activity in iPSCs [2]. Disrupting the proof-reading domain of the nuclear gene polymerase γ, which controls mtDNA replication, causes an accelerated accumulation of mtDNA mutations in a premature aging Mutator mouse model. These mtDNA mutations impact mitochondrial function with age, causing Mutator mice to suffer from weight loss, cardiomyopathies, age-related muscle wasting, fur graying, and other phenotypes that mimic human aging [3,4]. Prior work from the Suomalainen group showed that high mtDNA mutational loads in neural stem cells (NSCs) from Mutator mice do not result in a respiratory defect, but lead to oxidative phosphorylation dysfunction in adult neurons later in life [5]. MtDNA mutations in iPSCs or NSCs do not have the same adverse effects on oxidative phosphorylation activity as in other cell types, likely due to the heavy reliance of these stem cells on glycolysis for energy metabolism [6]. However, mtDNA mutations negatively affect the survival and proliferative abilities of stem cells, possibly due to alternative signaling pathways, such as the generation of reactive oxygen species [5]. It remains mysterious how a tRNA Leucine$^{(UUR)}$ mutation selectively impairs complex I in post-mitotic neurons when it is needed for the translation of all mitochondrial genes.

Neurons are complex specialized cell types categorized by location and by the type of neurotransmitters they release. Often, this view itself is simplistic; for example, different subtypes of dopaminergic neurons express different calcium-binding proteins and have distinct baseline neuronal firing oscillations. This is important because different disruptions in mtDNA integrity cause divergent neuroanatomical susceptibilities in the central nervous system [7]. Knocking out the function of complex III or complex IV in the same

subset of neurons expressing calcium/calmodulin-dependent protein kinase IIα(CaMKIIα) causes distinct patterns of neurodegeneration, resulting in dissimilar phenotypical consequences [8]. While future work will explore how different neuronal subtypes are dependent on mitochondrial function, it is noteworthy that Hämäläinen *et al.* [2] report that mtDNA mutations cause distinct types of mitochondrial dysfunction and compensation mechanisms that are unique to neurons.

Pharmacological and genetic knockout models that dissipate the mitochondrial membrane potential ($\Delta\psi m$) have supported the idea that Parkin, an E3 ubiquitin ligase, is recruited to dysfunctional mitochondria to target the whole organelle for autophagic engulfment and removal — a process termed mitophagy [9]. Hämäläinen *et al.* [2] demonstrate that Parkin recruitment and LC3 lipidation (a protein modification that indicates the induction of autophagy) specifically target the faulty complex I components for removal in m.3243A>G differentiated neurons. In agreement with this finding, specific respiratory complex proteins are subject to selective turnover in *Drosophila* brain mitochondria and this turnover is impeded in Parkin- and autophagy-deficient fly models [10]. Owing to the high respiratory demands of neurons for survival and physiological function, the complete removal of dysfunctional mitochondria may be energetically costly. In fact, cells attempt to compensate for inherited oxidative phosphorylation defects by an increase in mitochondrial proliferation [11]. It would be paradoxical to generate new mitochondria for destruction, so the selective removal of damaged oxidative phosphorylation complexes from otherwise functional mitochondria may yield a refined quality control mechanism that may also occur in other cell types harboring mtDNA mutations. In support of this idea, differentiated neurons generated from heteroplasmic iPSCs harboring mtDNA mutations in the COXI and ND5/ND6 genes disrupting complex IV and complex I, respectively, were able to maintain $\Delta\psi m$, even in the context of a severe loss of oxidative phosphorylation [12]. Thus, the depolarization of mitochondria cannot be the only way to alert the cell that mitochondria are dysfunctional. Consistent with this idea, accumulation of misfolded proteins inside mitochondria can trigger Parkin-mediated mitophagy without membrane depolarization [13]. How damaged subsets of mitochondrial proteins can be segregated for selective disposal remains to be elucidated.

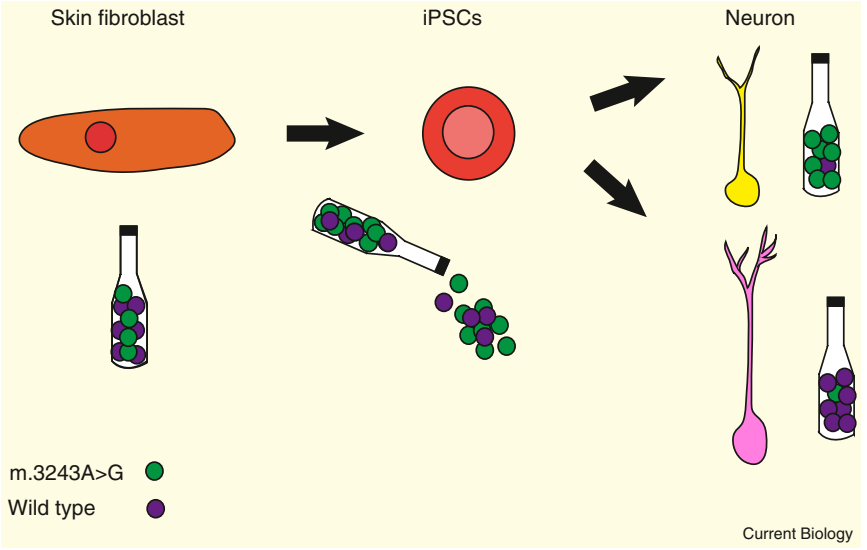

Figure 1. mtDNA bottleneck for tRNA mutations during iPSC differentiation.

Patient skin fibroblasts harbor a mutant mtDNA content of approximately 30%. During induced pluripotency, mtDNA copy number rises, shifting heteroplasmy to reach levels closer to homoplasmy in the direction of either mostly wild-type or mostly mutant molecules. The shift towards homoplasmy is permanent after differentiation.

Finally, this study shows that reprogramming of somatic cells to iPSCs segregates heteroplasmic mtDNA mutations differently than in somatic cells, as the m.3243A>G mutation preferentially shifts towards homoplasmy, populating iPSCs with either mainly mutant or mainly wild-type mtDNA molecules (Figure 1) [2]. It appears that cells undergo a genetic bottleneck during dedifferentiation, similar to mtDNA in the female germ line during oogenesis [14]. Contrary to the belief that mtDNA levels are reduced to generate a physical genetic bottleneck to preferentially select one mtDNA variant over another, Hämäläinen *et al.* [2] show that mtDNA levels are increased in iPSCs harboring mtDNA mutations during this reprogramming [14]. This new study confirms that mtDNA mutational selection occurs during the acquisition of stem cell fate, although we do not understand how this occurs. This genetic selection and increase in mtDNA levels appears to differ in iPSCs with wild-type mtDNA, as the levels of mtDNA drop as mitochondrial content is reduced, owing to the lack of demand during anaerobic metabolism [15]. These data also could explain the severe mitochondrial defects found in healthy aged human colon crypts that arise from mtDNA mutations in the crypt stem cells, which pass these mutations to their differentiated progeny [16]. Heart, skeletal muscle, and the central nervous system are the most affected tissues in mitochondrial disease patients. It was thought that, due to their post-mitotic state and low cell turnover, these tissues retained high levels of mutant mtDNA. Given the findings reported in this new study, it is possible that the regenerative stem cell pools in these tissues could also contribute to mtDNA mutant load. Yet, it is important to verify that this genetic bottleneck does not only occur during experimental reprogramming and that this heteroplasmic shift occurs in natural stem cell pools.

The work of Hämäläinen *et al.* [2] provides us with a deeper understanding of mtDNA biology in different cell types and gives insight into how mtDNA mutations are transmitted and lead to pathophysiological consequences. This powerful model, using these m.3243A>G iPSCs, can be used to investigate whether other post-mitotic cell types, such as skeletal muscle, also

show distinct phenotypes from the parental fibroblast line.

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Gene Regulation: When Analog Beats Digital

Why do some genes seem to respond in a 'digital', on/off manner to a graded signal, while others produce an 'analog', graded response? A new study suggests that the DNA-binding properties of transcription factors can strongly influence the response patterns of gene networks.

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Cells frequently need to change their gene expression profiles in response to external stimuli such as environmental stresses or developmental patterning signals. This is accomplished mainly through *cis*-regulatory DNA sequences, which contain binding sites for transcription factors, proteins with gene regulatory activities and binding preferences for specific DNA motifs. Signals received by the cell alter the abundance or activity of certain transcription factors, which directly activate or repress expression of specific target genes. Some signals require a sharp on/off response when a threshold has been reached, while others call for a graded response that is proportional to the intensity of the signal. A new study reported in this issue of *Current Biology* by Stewart-Ornstein *et al.* [1] addresses how these different transcriptional responses can be encoded in the genome.

Many transcription factors have long been known to bind DNA cooperatively, and/or to activate transcription in a highly synergistic manner, by diverse *in vitro* and *in vivo* assays [2–8]. Synergy among transcriptional activators is often essential for describing precise patterns of gene expression in multicellular organisms [9–12]. For example, in the *Drosophila* embryo, the Bicoid morphogen is proposed to create sharp boundaries of target gene expression through its high transcriptional cooperativity [5,7,13] (but see [14] for a fascinating update to that story). Countless developmental enhancers have been shown to rely on cooperative activation to integrate graded signals into seemingly 'digital' on/off patterns of gene expression [10,15].

Sometimes, however, a proportional response is called for. For example, the expression levels of many yeast genes are linearly correlated with growth rate [16]. Cell–cell signaling pathways, such as the pheromone response system in yeast and developmental patterning pathways in animals and plants, often employ negative feedback mechanisms to produce transcriptional responses in proportion to the intensity of the signal [17,18]. Cellular responses to stress and other environmental inputs are also typically 'analog' in the sense that the level of transcriptional response increases with the amount of stimulus. A 2010 study of the transcription factor NF-κB suggested that non-cooperative DNA binding to clusters of sites in target enhancers underlies its ability